Foraging mode: a factor affecting the susceptibility of spiders (Araneae) to insecticide applications

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Abstract: Field experiments have revealed that some species of spiders are more sensitive to insecticides than others. Among many factors influencing their susceptibility, foraging mode seems to play an important role. Aspects of foraging mode that appear to be relevant are whether the spider is diurnal or nocturnal, a hunter or a web-maker. Six spider species, *Araniella opisthographa, Clubiona neglecta, Dictyna uncinata, Pardosa agrestis, Philodromus cespitum* and *Theridion impressum* were used in the study. *P agrestis* and *P cespitum* are diurnal hunters that may come into direct contact with insecticide. *C neglecta* is nocturnal and so is exposed to residues only. The remaining three species are web-makers building webs that vary in the extent to which they can protect the spider from direct spray. The effect of sprays was tested under laboratory conditions (Potter tower) with three commercial insecticides, an insect growth regulator (hexaflumuron), a selective organophosphorus (phosalone) and a non-selective pyrethroid insecticide (permethrin) using a four-day exposure period. Data were analysed using bootstrap method and randomization tests. The results obtained showed that hunting spiders were more susceptible to the insecticides tested than web-makers (in their webs). Diurnal hunting spiders (*Philodromus* and *Pardosa*) were severely affected only by permethrin. A high mortality was observed for the nocturnal hunter, *Clubiona*, after application of phosalone and permethrin. This species appears to be very sensitive to residues of both insecticides. Comparing the effect on web-making spiders, with and without webs, it was observed that the sparse orb-web of *Araniella* did not protect its owner at all, but the dense cribellate and frame-webs of *Dictyna* and *Theridion*, respectively, reduced the mortality caused by permethrin significantly in comparison with specimens without webs. Of other factors studied, posture (normal and upside-down position) did not influence the susceptibility. Mortality increased slightly with body size after permethrin application.

Keywords: spiders; Araneae; insecticide; laboratory test; susceptibility; foraging mode; bootstrap; randomization test

1 INTRODUCTION

Integrated Pest Management aims to avoid harming natural enemies by employing insecticides of selectively low toxicity.[1] Under such conditions the density of natural enemies can increase, resulting in improved pest control. Information on the effect of insecticides on beneficial arthropods is rather scarce. The IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'[2,3] and other researchers[4,5] aim to remedy this deficiency by making extensive tests with commercial formulations (at recommended application rates) on selected natural enemies. Although such data are valuable, their application is limited because susceptibility to insecticides differs among species of beneficial organisms. In particular, susceptibility varies between species of spiders,[6] one of the most abundant and diverse groups of natural enemies occurring in agrobiocenoses.[7]

It was shown in field experiments (eg commercial orchards) that some species of spiders are less susceptible to certain insecticides than others.[8] Specifically, ambush spiders (Philodromidae) were found to be more sensitive to insecticide applications than web-making spiders (Theridiidae). This was suggested to be related to a number of behavioural factors.[9,10] A large variation in the response of spiders to insecticides was found even in laboratory tests carried out under standardized conditions. The authors attributed this variation to the behaviour of tested specimens and leaching or adsorption.[6,11] Taking this into account,

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Contract/grant sponsor: Grant Agency of the Czech Republic; contract/grant number: 522/97/0271
(*Received 23 September 1998; revised version received 6 May 1999; accepted 25 June 1999*)

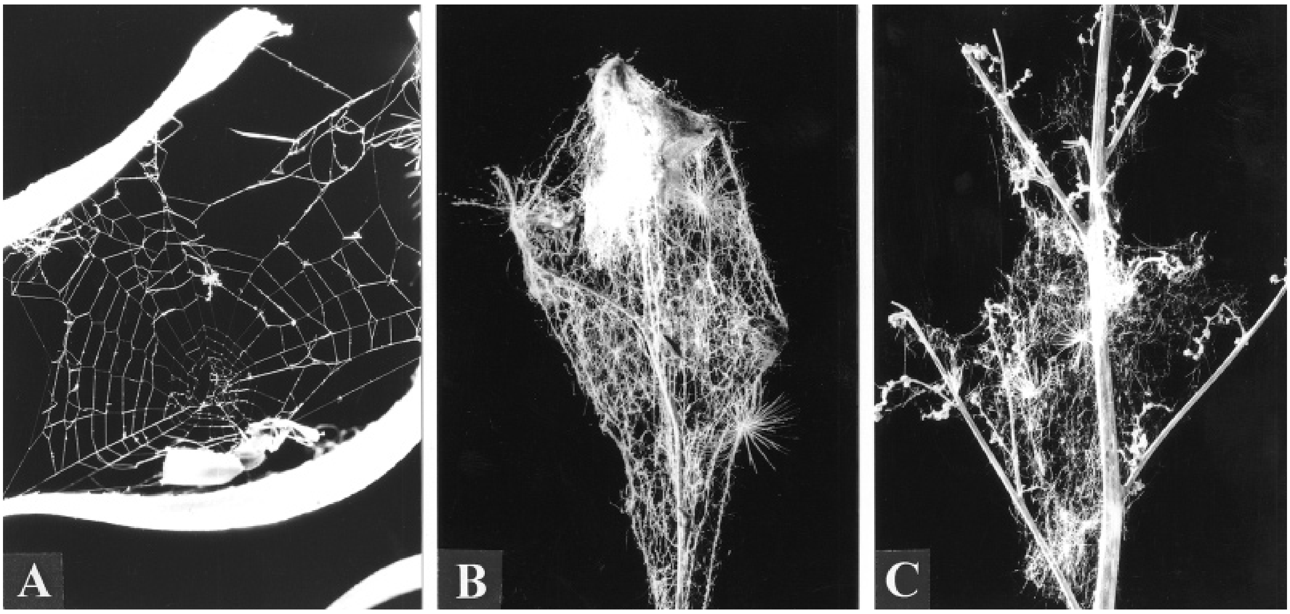

Figure 1. Examples of webs. A – an orb-web of *Araniella opistographa;* B – a cribellate frame web of *Dictyna uncinata;* C – a tangled frame web of *Theridion impressum.*

Hassan *et al.*[3] suggested further testing, using semi-field and field methods, when a pesticide is found to be harmful in the laboratory.

Behaviour and foraging mode apparently influence species' susceptibility. For example, webs are known to be efficient collectors of sprayed pesticides.[12,13] They can protect the spider from direct contact with an applied insecticide or can endanger species that recycle their web. The scope of the present study was to investigate how the foraging mode affects species' susceptibility to some commercially available insecticides, mimicking natural conditions as far as possible. Aspects of foraging mode that appear to be relevant are whether the spider is diurnal or nocturnal, a hunter or a web-maker. The following dominant spider species of agricultural habitats in the Czech Republic were studied. *Araniella opistographa* (Kulczynski) is a diurnal orb-weaver (Fig 1A) constructing quite a small orb-web among leaves. It does not built any shelter but sometimes hides beneath leaves; therefore it is expected to be directly affected by sprays. *Clubiona neglecta* O P Cambridge is a nocturnal hunter which spends the daylight hours in a silk cell under stone or bark.[14] This spider thus comes into contact only with residues. *Dictyna uncinata* Thorell, spins its cribellate web (Fig 1B) on dry heads of vegetation. It is diurnal but often hides in its web so should be somewhat protected from spray. *Pardosa agrestis* (Westring) is a diurnal hunter running on the ground, and is thus exposed to direct spraying. *Philodromus cespitum* (Walckenaer) is a diurnal ambush spider hunting on leaves which is also exposed to direct spray. *Theridion impressum* (L) Koch is a diurnal frame-builder (Fig 1C). It spins a tangled three-dimensional web incorporating a retreat where it hides. However, this species often displays itself on the web and thus may come in contact with sprayed chemical.

2 EXPERIMENTAL METHODS

2.1 Treatment of spiders

Spiders used in the experiment were collected in the field, near the Research Institute of Crop Production, Prague, Czech Republic in April–May 1998. Some spiders (*Araniella* and *Philodromus*) were collected by beating the low branches of trees over a collecting tray, others by hand-collecting on the ground (*Clubiona* and *Pardosa*), and from dead heads of vegetation (*Theridion* and *Dictyna*). The latter two species were collected both with and without their webs. The spiders were kept at constant conditions of 20 ($\pm$1) °C, 60% RH, with a 14:10 h light:dark regime. Twice a day, in the morning and in the evening, they were sprayed with water. No food was given to the spiders during the experiment. All specimens were of uniform age, ie juvenile. Pre-mature spiders were used because most insecticides are applied in spring when spiders are immature.

Forty specimens of each species were used. The hunting spiders, *Clubiona* (in a silk cell), *Pardosa*, and *Philodromus*, and the web-making spiders, *Araniella, Dictyna*, and *Theridion* without webs were kept singly in a Petri dish (diameter 8 cm) to avoid cannibalism. The dish was covered with a wire mesh (1 mm) to provide ventilation. The bottom of the dish was covered with a thin (1 mm) sand layer. Web-makers with webs were kept singly in a plastic box ($10 \times 8 \times 6$ cm) covered with wire mesh.

Three insecticides were used in this study: an insect growth regulator (IGR), hexaflumuron ('Consult'

Species		Posture[a]	Body size (mm) (±SE)[b]	Web density (%)
Araniella	Without web	−	3.17 (±0.39)	−
	With web	−		7.93
Clubiona			4.07 (±0.45)	−
Dictyna	Without web	+	2.92 (±0.29)	−
	With web	+		26.7
Pardosa		+	4.71 (±0.51)	−
Philodromus		−	3.46 (±0.40)	−
Theridion	Without web	−	2.41 (±0.33)	−
	With web	−		18.5

Table 1. Factors possibly affecting the mortality of spiders

[a] + = normal, − = upside-down.
[b] 20 specimens.

100EC, Dow Elanco) which is a non-systemic insecticide (chitin synthesis inhibitor); an organophosphorus insecticide, phosalone ('Zolone' 35EC, Rhône-Poulene) which is a non-systemic acaricide with both contact and stomach action, selective of beneficial arthropods; a pyrethroid, permethrin ('Ambush' 25EC, Zeneca) which is a non-systemic non-selective (ie effective against a wide range of pests) insecticide.[15] The insecticide formulations were diluted in water to the desired AI concentration, specifically 0.35 g $litre^{-1}$ hexaflumuron, 3.6 g $litre^{-1}$ phosalone, and 0.7 g $litre^{-1}$ permethrin. Those concentrations are recommended for orchard applications in the Czech Republic.[16]

The insecticides were applied to 30 specimens of each species in a Potter tower. The toxicity tests were carried out in the afternoon, between 1 and 4 pm, on the day the spiders were collected, except for *Araniella* with its web. Specimens of this species underwent testing after construction of an orb-web on offered pieces of branches. This usually happened after 24 h. The dosage used (2 ml per spider) was evaluated from the quantity of spray used in the field applications (1000 litre ha^{-1}). After application the containers were put in a well-ventilated place to speed up evaporation of the applied insecticide, so that the containers were dried in 10 min. Ten specimens of all species were used as controls. These were sprayed with water instead of insecticide. Mortality of all specimens was recorded during four days after treatment. Control mortalities were zero for *Philodromus, Pardosa, Dictyna* and *Theridion*, 10% for *Clubiona*, and 20% for *Araniella*.

The total body size of 20 individuals of each species was measured, after treatment, under a binocular stereomicroscope. Density of *Araniella, Dictyna* and *Theridion* webs was estimated as follows: a picture of a web was taken, then digitized using a scanner and finally the percentage of web area per cm^2 was computed using image analysis software (SIGMASCAN).

2.2 Statistical analyses

As the observed data did not meet some statistical assumptions, non-parametric statistics were applied. All analyses were conducted using the Resampling Stats package.[17] The bootstrap method adapted from Simon[18] was used for the comparison of two mortality values (1000 paired samples were repeatedly drawn from a generated universe of total mortality, and the probability was estimated as the proportion of trials in which the simulated difference between samples was larger than or equal to the observed one). A randomization test[19] was used in comparison of two or three samples. There were two variables measured for each species (Table 1). The continuous variable (body size) was analysed by randomized regression after Manly[19] (randomly reallocating the Y values to the X). The discrete variable (posture, ie normal or upside-down position) was analysed using a two-sample randomization test.[19] Because the species studied showed non-consistent control mortality, corrected mortality (=sprayed − control) is treated further in the text.

3 RESULTS

The mortality of sprayed hunting spiders, and of web-makers with and without webs was compared with control mortality of the particular species. The bootstrap analysis showed that application of hexaflumuron caused a reduction of 0 to 23%, but for no species was the application significantly toxic (Table 2). The highest sensitivity to this insecticide was found for *Dictyna* (without web), and the lowest for *Theridion* (with web). Phosalone caused a mortality of 3 to 37% and was significantly detrimental for *Clubiona, Pardosa, Araniella* (with and without web), and *Theridion* (without web). The least susceptible to this insecticide was *Philodromus*. Permethrin was very toxic to all spider species, causing a mortality of 30 to 90%. The most resistant species to this insecticide was *Theridion* and the least *Araniella, Clubiona*, and *Philodromus*. Averaged over all taxa, permethrin was the most toxic insecticide (81.8 (±6.4)% mortality on average), followed by phosalone (25.6 (±5.1)%), and hexaflumuron (12.8 (±2.9)%). The difference between these three insecticides was highly significant ($P < 0.001$, three-sample randomization test, 4999 simulations).

Hunting spiders (*Clubiona, Pardosa, Philodromus*) were on average more severely affected than web-makers with webs. However the difference was significant only after application of permethrin (Table

Table 2. Corrected mortality of studied spiders four days after treatment

Species		Mortality (%)[a] Hexaflumuron ('Consult' 100EC, 0.35g AI litre^{-1})	Phosalone ('Zolone' 35EC, 3.6g AI litre^{-1})	Permethrin ('Ambush' 25EC, 0.7g AI litre^{-1})
Araniella	Without web	10	33.3	73.9
	With web	13.3	26.7	66.7
Clubiona		6.7	36.7	90
Dictyna	Without web	23.3	20	90
	With web	10	16.7	50
Pardosa		10	26.7	93.3
Philodromus		20	3.3	90
Theridion	Without web	6.7	33.3	53.3
	With web	0	10	30

[a] Underlined values are significantly different from the control mortality at the 10% level and double-underlined at the 5% level (results of the bootstrap analysis).

3). Among hunting spiders, both diurnal (*Pardosa, Philodromus*) and nocturnal species (*Clubiona*) showed similar susceptibilities to all insecticides applied.

For web-making spiders, mortality of sprayed specimens with and without their webs was compared using the bootstrap method. In the orb-weaver *Araniella*, mortalities of specimens on webs were similar to those without, after application of all insecticides (all $P > 0.25$). In the cribellate spider, *Dictyna*, individuals without webs were always more affected than those hidden in their webs. However, a significant difference of 40% was observed only after permethrin spraying ($P < 0.001$). As for the frame-building spider, *Theridion*, the mortalities of specimens without webs were in all cases higher than those with webs. The difference of 23% was significant after both phosalone and permethrin applications ($P = 0.01$ and 0.05, respectively). The different susceptibilities of these species thus appear to be a function of web density, but a wider range of species (of different web structures) should be investigated to confirm this.

Two factors possibly influencing the studied species' susceptibility were noticed (Table 1) and tested as predictors of mortality. Posture (normal *versus* upside-down position with the ventral surface uppermost) did not seem to influence susceptibility (all $P > 0.30$, two-sample randomization test, 56 simulations) of hunting or web-making spiders. Body size had a certain effect for hunting spiders only after application of permethrin ($R = 0.73$, $P = 0.10$, randomized correlation, 720 simulations). Mortality was found to increase slightly with body size (Fig 2), as apparent from a linear regression of this relationship: $y = 35.3 + 14.5x$.

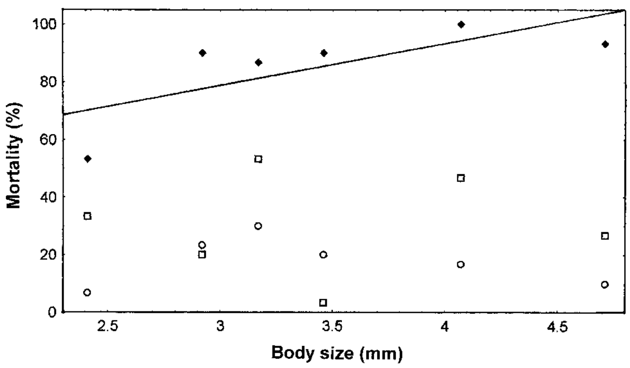

Figure 2. Relationship between body size and percentage mortality after application of (□) hexaflumuron, (○) phosalone, and (◆) permethrin. Linear regression is shown only for permethrin.

4 DISCUSSION

The spiders investigated all had different modes of foraging. Of the three hunting spiders, *Philodromus* and *Pardosa* are diurnal and may therefore be exposed directly to spray. *Pardosa* was severely affected by both organophopsphate and pyrethroid insecticides, while *Philodromus* was severely affected only by permethrin. In tree crowns *Philodromus* specimens presumably come more often into contact with insecticide than *Pardosa* on the ground. Thus this ambush spider might have evolved a tolerance to insecticides as already noted by Mansour and Nentwig.[6] Mortality of *Clubiona* specimens was fairly high after application of phosalone and permethrin. This spider species rests in a silk cell during the day, so is exposed only to residues. Nevertheless, even these insecticides were

Table 3. Comparison of mean mortalities for hunting (*Clubiona, Pardosa, Philodromus*) and web-making (*Araniella, Dictyna, Theridion*) spiders after application of tested insecticides

	Mortality (%) (±SE) Hunters	Web-makers with web	P^a
Hexaflumuron ('Consult' 100EC, 0.35g AI litre^{-1})	12.2 (±3.9)	7.8 (±3.9)	0.35
Phosalone ('Zolone' 35EC, 36g AI litre^{-1})	22.2 (±9.8)	17.8 (±4.8)	0.66
Permethrin ('Ambush' 25EC, 0.7g AI litre^{-1})	91.9 (±1.1)	48.9 (±10.6)	0.02

[a] Probability at which the two mean mortalities differ, estimated from the two-sample randomization test.

found to be very toxic, as was also shown by Mansour and Nentwig[6] and Mansour *et al*[20] for other species of spiders.

Among web-makers, three kind of webs are recognized in the species investigated here. The results showed that the orb-webs of *Araniella* do not protect the spider from spray because the webs are very sparse and more or less two-dimensional. A few threads can catch only a limited amount of sprayed compound. However, Pekár[21] observed that, in an apple orchard, this species survived even the application of non-selective insecticides. This is mainly a result of their behaviour. This species often hides under leaves and thus is somewhat protected from direct contact with the chemical. On the other hand, sensitivity of this species might be increased due to fact that like other cross-spiders it recycles its web.[12] Spiders of the family Araneidae thus may be affected not only by contact insecticides but also by insecticides with stomach action.

The web of the cribellate spider, *Dictyna*, is denser and not so stretched as that of *Theridion*, so it offers a better shelter. Moreover, this species does not expose itself on the web as often as *Theridion* and so it is even more protected. This was noticed particularly after permethrin application, where the mortality of *Dictyna* specimens decreased by about 40% in comparison with spiders hidden in webs. It can be concluded that the webs of *Theridion* and *Dictyna* provide efficient protection to their owners, so these species of spiders can not only survive but even become abundant in agrobiocenoses under strong insecticide pressure.

Vertical location of webs should influence survival of web-making spiders. Webs located near ground will receive less spray than those built in e g a tree crown. All web-making species studied here occur as frequently on vegetation near the ground as in trees. Specimens near the ground thus might be a pool for dispersion into tree crowns during and after spraying. Undersowing of suitable plantings in orchards may, therefore, enhance spider density.

A great variation was recorded in susceptibility of spiders to studied insecticides. This increased in the following order: hexaflumuron < phosalone < permethrin. As suggested by many authors, differences in spider susceptibility are affected by many factors other than behaviour. In this study the effect of posture and body size was tested. Upside-down posture was believed to be important since, in this position, sprayed chemical can easily reach the book lungs. However, the results do not support the influence of this factor. Body size had only negligible effect as the mortality increased slightly with body size. However, further experiments are required to confirm impact of this factor.

The insecticides tested were selected to represent different types of common, widely available insecticides. All were non-systemic insecticides, and one was selective. Hexaflumuron was expected to be harmless to all spider species, as also found by Pekár[22] in a field experiment. The results support this suggestion, as in none of the species studied did application of this insecticide cause significant mortality over a four-day exposuure period. Phosalone, considered as selective of many beneficial arthropods,[15] was also expected to be harmless. However, some authors have shown that it is in fact of variable toxicity, from harmful to harmless for various natural enemies including spiders.[2–4] Results of this study are consistent with published data; two hunting spiders (*Clubiona* and *Pardosa*) and two web-makers without webs (*Araniella* and *Theridion*) were severely affected by this insecticide. The non-selective pyrethroid, permethrin, was expected to be harmful to all studied species as shown by Hassan *et al.*[2] However, *Theridion* without webs showed a surprisingly high resistance to this insecticide (only 50% mortality). It might result from a resistant strain selected during the many years of non-selective spraying practised in the Czech Republic. However, a satisfactory answer can be given only after more research on this phenomenon.

SUMMARY

1. Hunting spiders were on average more susceptible than web-makers.
2. Although nocturnal *Clubiona* spiders are not exposed to direct spray, specimens of this species were severely affected by residues.
3. Orb-web of *Araniella* did not provide efficient protection for the spider.
4. Cribellate and frame webs of *Dictyna* and *Theridion*, respectively, offered quite efficient shelter for the species.

ACKNOWLEDGEMENTS

I am very grateful to Dr KD Sunderland and two anonymous referees for comments on the manuscript and alterations to the English. The research was supported by the Grant Agency of the Czech Republic (grant no 522/97/0271).

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